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Proposed Science Policy: PPARα-mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessment

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Outline

- Hepatic Tumors
  - Establishing PPARα Mode of Action in Adult Rodents
  - Development of PPARα Activity and Responses to PPARα Agonists in the Fetus and Neonate
  - PPARα Agonism in Non-human Primates and Humans
  - Data Needs
  - Proposed Science Policy
- Leydig and Pancreatic Acinar Cell tumors

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History of PPARα Mode of Action (MOA) Evaluation

- 1994 - IARC (Technical Report No. 24)
  - Peroxisome Proliferation and its Role in Carcinogenesis
- 1995 - ILSI/HESI (Regul. Toxicol. Pharmacol.1998)
  - Do Peroxisome Proliferative Compounds Pose a Hepatocarcinogenic Hazard to Humans?
- 2003 – ILSI/RSI (Crit. Rev. Tox. 2003)
  - Report: PPARα Agonist-induced Rodent Tumors: Mode(s) of Action and Human Relevance

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Definitions and Approaches to Evaluate a MOA

"Mode of Action"

is contrasted with

"Mechanism of Action"

implies a more detailed molecular description of events

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MOA Framework (USEPA, 1999; IPCS, 2001)

Questions to be addressed:

- Identify key events
- Dose-response relationship
- Temporal associations
- Biological plausibility & coherence
- Strength, consistency & specificity
- Other modes of action

{Relevance to humans}

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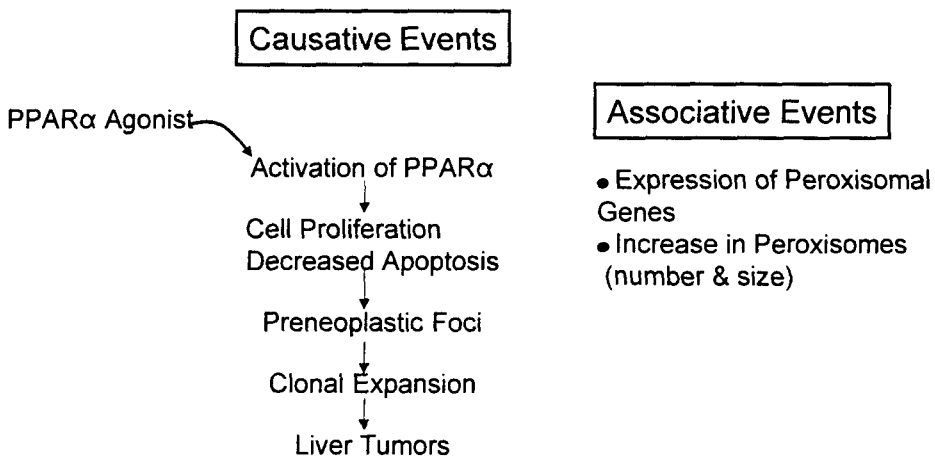
Key Events in the PPARα MOA for Rodent Hepatocarcinogenesis



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Rodent Response to PPARα Agonists

- *In vitro* Studies with Rodent Hepatocytes
  - Effects following PPARα agonist exposure (*e.g.* clofibrate, MEHP, WY14643)
    - Reporter construct activation
    - Peroxisome proliferation
    - Peroxisomal enzyme activity (*e.g.* Acyl CoA oxidase and Pal CoA oxidase)
    - Replicative DNA synthesis
    - Suppression of apoptosis

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Rodent Response to PPARα Agonists

- *In vivo* studies: Effects in Wild Type Mice and/or Rats
  - Peroxisome proliferation
  - Peroxisomal enzyme activity
  - Replicative DNA synthesis
  - Apoptosis suppression
  - Hepatocellular proliferation
  - Selective clonal expansion
  - Liver weight increases
  - Liver tumors

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Rodent Response to PPARα Agonists

- *In vivo* Studies: Effects in PPARα-null Mice
  - No hepatic peroxisome proliferation
  - No acyl CoA oxidase induction
  - No replicative DNA synthesis
  - No apoptosis suppression
  - No increased liver weights
  - No hepatic neoplasms

Establishing PPARα MOA in Rodents

Conclusion

There is sufficient weight of evidence to establish the MOA for PPARα agonist-induced rodent liver tumors.

Ontogeny of PPARα Features in Rodents

- Late rodent fetal development (*i.e.* gestation day 15 or later)
  - Expression of PPARα gene (low relative to adults)
  - Assemblage of peroxisomes
  - Peroxisomal enzyme activity
- PPARα features comparable to adults after birth

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Response of the Young to PPARα Agonists

- Peroxisomal features are comparable to adults at birth
- Respond like adults to PPARα agonists
- Response comparable between young and adults

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Gestational Exposure

Wistar rats exposed via the diet to Clofibrate for 7 days.

Peroxisomal Enzymes	GD 8-15 Exposure		GD 19-21 Exposure	
	Fetal	Maternal	Fetal	Maternal
Pal CoA oxidase	≈4X	≈ 3X	≈6-8X **at birth**	≈ 5X
Catalase	No increase	≈2X	≈3X **at birth**	≈ 2X

Cibelli, *et al.* 1988

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Gestational Exposure

Wistar rats exposed to Clofibrate via the diet for 7 days

Peroxisomal Parameters	End of Exposure				
	GD 13	GD 15	GD 19	GD 21	Newborn
Numerical density	Not present	≈1.5X	≈ 1.8X	≈2.5X	≈ 2.4X
Volume density	Not present	≈3.4X	≈ 3.9X	≈4.3X	≈ 4.6X

Stefanini, *et al.* 1989

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Direct Exposure

Neonatal, Weanling, Young Adult and Adult rats gavaged with 100 mg/kg/day DEHP

Parameter	Neonatal/Weanling			Young Adult/Adult	
	PND 6-10	PND 14-18	PND 21-25	PND 42-46	PND 86-90
Pal CoA oxidase	≈3X	≈7X	≈2X	≈2.5X	≈4X
Carnitine acetyl transferase	≈2.7X	≈7.8X	≈2.4X	≈3.6X	≈4.4X
Liver weights	N/A	≈1.2X	N/A	≈1.1X	≈1.1X

Dostal *et al.*, 1987

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Direct Exposure

Weanling, Young Adult, and Adult F344 Rats Gavaged with Clofibrate

Parameters Evaluated	Weanling		Young Adult		Adult	
	4 Weeks Old		8 Weeks Old		12 Weeks Old	
	Male	Female	Male	Female	Male	Female
Volume Density	34%	68%	379%	136%	557%	169%
Pal CoA oxidase	≈2X	≈1.4X	≈5.8X	≈1.5X	≈10.7X	≈3X

Yamamoto, 1996

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Indirect Exposure

Neonatal/Weanling Rats from Dams Exposed via the Diet

- Neonatal/Weanling response to PPARα agonists comparable to adults.
  - DHAP-AT
  - Pal CoA oxidase activity
  - Peroxisomal β-oxidation
  - Numerical density
  - Liver weights

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Response of the Young to PPARα Agonists

- Peroxisomal features are comparable to adults at birth
- Respond like adults to PPARα agonists
- Response comparable between young and adults

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PPARα Agonism in the Young Rodent

Conclusion

Any conclusions regarding this mode of action in adult rodents would also apply to the young.

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Non-human Primate Response to PPARα Agonists

- *In vitro* studies
  - Non-human primate hepatocyte response
    - No peroxisome proliferation
    - No increased peroxisomal enzyme activity
    - No increased replicative DNA
    - No apoptosis suppression

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Non-human Primate Response to PPARα Agonists

- *In vivo* studies
  - Non-human primates response to PPARα agonist exposure
    - Slight increase in peroxisome proliferation, liver weight, and hepatocellular hypertrophy
    - Minimal increase in peroxisomal enzyme activity
    - No increase in replicative DNA
    - No evidence of liver tumors

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Human Response to PPARα Agonists

- *In vitro* studies
  - Human hepatocyte response
    - No peroxisome proliferation
    - No increased peroxisomal enzyme activity
    - No increased replicative DNA
    - No apoptosis suppression

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Human Response to PPARα Agonists

- *In vivo* studies
  - Liver biopsies from patients receiving hypolipidemic drugs (*e.g.* gemfibrozil, clofibrate or ciprofibrate) for 2 months to 8 years
    - Minimal peroxisome proliferation

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Response to PPARα Agonist Species Differences

Compound	Rodents	Non-human Primates	Humans	References
Beclobric acid	Yes	No	No	Blaauboer *et al.*, (1990)
Ciprofibrate	Yes	No	No	Allen *et al.*, (1987); Foxworthy *et al.*, (1990); Perrone *et al.*, (1998)
Clofibrate	Yes	No	No	Allen *et al.*, (1987);
Clofibric Acid	Yes	No	No	Bichet *et al.*, (1990); Blaauboer *et al.*, (1990); Butterworth *et al.* (1989); Elcombe *et al.* (1996); Richert *et al.* (1996)
Fomesafen	Yes	No	No	Smith & Elcombe (1989); Elcombe *et al.* (1996)

•Response defined as changes in enzyme activity (*e.g.* pal CoA oxidase), organelle proliferation and/or changes in DNA labeling indices

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Response to PPARα Agonist Species Differences

Compound	Rodents	Non-human Primates	Humans	References
Trichloroacetic acid	Yes	Not determined	No	Elcombe (1985); Elcombe (1996)
WY14643	Yes	Not determined	No	Butterworth (1989)
MEHP	Yes	No	No	Bichet *et al.*, (1990); Butterworth *et al.* (1989); Dirven *et al.* (1993); Elcombe & Mitchell (1986)
Methylclofenapate	Yes	Not determined	No	Elcombe & Styles (1989); Elcombe *et al.* (1996)

Extracted from Doull's *et al.*, 1999

- Response defined as changes in enzyme activity (*e.g.* pal CoA oxidase), peroxisome proliferation and/or changes in DNA labeling indices

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Human Response to PPARα Agonists

- Limited data from human clinical studies do not indicate a potential for liver tumor formation
  - Helsinki Heart Study and WHO study
  - Patients treated with hypolipidemic drugs or placebo
    - Liver cancer rates comparable between the two groups

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Basis for Differential Response of Humans to PPARα Agonists

- *In vitro* studies
  - In humans, PPARα expression is 10-fold lower than in rodents
  - Human peroxisome proliferator response element (hPPRE) may differ from the rodent PPRE
    - PPARα responsive genes containing a hPPRE do not respond to PPARα agonist exposure
    - HepG2 cells transfected with hPPARα (at levels comparable to rodent PPARα) do not exhibit acyl CoA oxidase activity increases after fibrate exposure
    - Acyl CoA oxidase hPPRE is not responsive to PPARα agonists.

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Key Events in PPARα MOA

- Plausible in humans
  - Activation of PPARα
- Not likely in humans
  - Expression of peroxisomal genes
  - Peroxisome proliferation
  - Perturbation of cell proliferation
  - Perturbation of apoptosis
  - Tumor formation

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PPARα Agonism in Humans and Non-human Primates

Conclusion

Although humans possess a functional PPARα, the weight of evidence indicates that humans (and non-human primates) appear to be refractory to the key events associated with PPARα agonist-induced hepatocarcinogenesis

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Data Set to Demonstrate a PPARα MOA

- Evidence of PPARα agonism
  - *e.g. in vitro* reporter gene assay
- *In vivo* evidence demonstrating dose-response and temporal concordance between precursor events and liver tumor formation
  - Evidence of increase in the number and size of peroxisomes
  - Increased acyl CoA oxidase activity
  - Hepatic cell proliferation

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Additional Data to Support Weight of Evidence Analysis for PPARα MOA

- Hepatic CYP4A1 induction
- Increased pal CoA oxidase activity
- Hepatocyte hypertrophy
- Increased liver weights
- Decreased apoptosis
- Increased microsomal fatty acid oxidation
- Increased hydrogen peroxide formation

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Proposed Science Policy

- When liver tumors are observed in long-term studies in rats and/or mice and:
  - Data are sufficient to establish that the liver tumors are a result of a PPARα agonist MOA
  - Other potential MOAs (*e.g.* mutagenicity, cytotoxicity) have been evaluated and not operative

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Proposed Science Policy

Conclusion

Hepatic effects in rodents that are the result of PPARα agonism **should not** be used to characterize potential human hazard

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Leydig Cell and Pancreatic Acinar Cell Response to PPARα Agonists

- Tumor triad
  - Liver, pancreatic acinar, and Leydig cell tumors
  - Nine PPARα agonists linked to tumor triad

    Clofibrate, DEHP, fenofibrate, gemfibrozil, HCFC-123, methyl clofenapate, ammonium perfluorooctanoate, tibric acid and WY14,643

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Leydig Cell and Pancreatic Acinar Cell Response to PPARα Agonists

- Leydig cell tumor formation
  - Two proposed MOAS
    - Induction of hepatic aromatase activity leading to an increase in serum estradiol level **or;**
    - Inhibition of testosterone biosynthesis
- Pancreatic acinar cell tumor formation
  - One MOA proposed
    - Decrease in bile acid synthesis and/or change the composition of the bile acid resulting in cholestasis

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Leydig Cell and Pancreatic Acinar Cell Tumors and PPARα Agonists

Conclusion

The evidence is inadequate at this time to support a linkage between PPARα agonism and formation of these tumor types.

Thus, it is presumed that chemicals in this subclass that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

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Charge to the Science Advisory Panel

- Establishing the PPARα MOA in rodent hepatocarcinogenesis
- Relative sensitivity of young and adult rodents
- Human relevance
- Data needed to establish the MOA is operative
- Other tumor types induced by PPARα agonists

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Charge to the Panel
Issue 1

Rodent PPARα Mode of Action for Hepatocarcinogenesis

OPPTS has concluded that there is sufficient weight of evidence to establish the mode of action for PPARα agonist-induced rodent hepatocarcinogenesis. It is proposed in the OPPTS document that PPARα agonists activate PPARα leading to an increase in cell proliferation, and a decrease in apoptosis, and eventually further clonal expansion of preneoplastic cells and formation of liver tumors. The key events in PPARα agonist-induced rodent hepatocarcinogenesis may be classified as either causal (required for this MOA) or associative (marker of PPARα agonism).

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Charge to the Panel
Question 1

Please comment on the weight of evidence and key events for the proposed mode of action for the PPARα agonist-induced rodent hepatocarcinogenesis. Please comment on the adequacy of the data available to identify the key events in the PPARα MOA. Discuss whether uncertainties and limitations of these data have been adequately characterized.

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Charge to the Panel
Issue 2

Relative Sensitivity of Fetal, Neonatal, and Adult Rodent

OPPTS has provided a review of the ontogeny of PPARα expression and peroxisomal assemblage during fetal and postnatal development in rodents as well as an analysis of the available data evaluating effects on peroxisome proliferation, peroxisomal enzyme activity, and liver weights following exposure to PPARα agonists during fetal and postnatal development in rats and mice. Based on this analysis, OPPTS concluded that fetal and neonatal rats do not exhibit an increased sensitivity to PPARα agonist-induced hepatocarcinogenicity relative to the adult rodent. Therefore, any conclusions regarding this mode of action in adult rodents would also apply to the young.

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Charge to the Panel
Question 2

Please comment on the weight of the evidence approach and mechanistic data used to support this conclusion.

Charge to the Panel
Issue 3
Human Relevance

OPPTS has provided an analysis of a variety of *in vitro* and *in vivo* studies on the key events pertaining to PPARα agonist-induced hepatocarcinogenesis with hamsters, guinea pigs, non-human primates, and humans. Based on the weight of the evidence, OPPTS concludes that although PPARα agonists can induce liver tumors in rodents and while PPARα is functional in humans, quantitatively, humans and non-human primates are refractory to the hepatic effects of PPARα agonists.

Charge to Panel
Issue 3

Therefore, OPPTS is proposing the following science policy:

When liver tumors are observed in long term studies in rats and mice, and 1) data are sufficient to establish that the liver tumors are a result of a PPARα agonist MOA and, 2) other potential MOAs have been evaluated and found not operative, the evidence of liver tumor formation in rodents **should not** be used to characterize potential human hazard.

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Charge to the Panel
Question 3

Please comment on the data and the weight of evidence regarding the hepatic effects of PPARα agonists in humans, and please comment on the proposed OPPTS science policy regarding human relevance.

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Charge to the Panel
Issue 4

Data Requirements

OPPTS has proposed a data set that would be sufficient to demonstrate that PPARα agonism is the mode of action for the induction of rodent liver tumors. The data set includes evidence of PPARα agonism (*i.e.* from an *in vitro* reporter gene assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The *in vivo* evidence should be collected from studies designed to provide data needed to show dose-response and temporal concordance between precursor events and liver tumor formation.

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Charge to the Panel
Question 4

Please comment in general on the proposed data set and particularly on its adequacy to demonstrate that a PPARα agonist-mediated MOA is operating in rodent hepatocarcinogenesis.

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Charge to the Panel
Issue 5

Other Tumors Induced by PPARα Agonists

Some PPARα agonists may also induce pancreatic acinar cell and Leydig cell tumors in rats and modes of action involving agonism of PPARα have been proposed. An in depth analysis of these tumors is provided in the 2003 ILSI technical panel report. Based on this analysis, OPPTS agrees that the data available are insufficient to support the proposed MOAs.

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Charge to the Panel
Issue 5

Thus, OPPTS is proposing the following science policy:

Given the limited evidence available to support that a chemical may induce pancreatic and Leydig cell tumors through a PPARα agonist mode of action, the evidence is inadequate to support a linkage between PPARα agonism and formation of these tumor types. Thus, it is presumed that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

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Charge to the Panel
Question 5

Please comment on OPPTS's conclusion that there is limited evidence that a chemical may induce pancreatic and Leydig cell tumors through a PPARα agonist mode of action, and OPPTS's proposed science policy regarding other tumors induced by PPARα agonists.

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